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RESEARCH ARTICLE

Molecular Epidemiology and Recycling of *Staphylococcus aureus* Resistant to Methicillin Among the Staff, Patients, and Surfaces in University Hospital in West Iran, Ilam

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Abstract: ***Background:*** *Staphylococcus aureus* is a human pathogen causing nosocomial infections and increased hospitalization and mortality among human communities. Methicillin-resistant *S. aureus* strains are considered a severe threat in nosocomial infections and cause complications in the remedy process of bacterial infections. In this study, 137 samples were collected from different departments, staff, and patients in Ilam hospital.

Methods: Eighty-eight samples of these strains were examined to test antibiotic resistance and diffusion. MIC (minimum inhibitory concentration) and PCR (polymerase chain reaction) were performed on the samples resistant to oxacillin. 36 (40.9%) strains were MRSA, and 52 (59.1%) isolates were MSSA. 44.4% of MRSA strains with IV SCC*mec* type.

Results: Fourteen different *spa* types were found using *spa* typing, of which the most abundant types were t037, t030, and t701, and three new types, including t15471, t15474, and t17470, were identified among the strains. The molecular analysis by MLST showed that the strains are classified into 11 different sequence types. Sequence type 239 and clonal complexes of 329 and 22 were dominant. ST239-*spa*t037-SCC*mec* III was also identified as the most frequent clone of MRSA. The most identified clones were MRSA ST239-*spa* t037-SCC*mec* III.

Conclusion: The results show the *spa-type* distribution between samples of patients, personnel, and surfaces, demonstrating MRSA circulation between patients and the environment. The results show the need to control environmental health.

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1. INTRODUCTION

Staphylococcus aureus is a human pathogen causing nosocomial infections and increased hospitalization and mortality among human communities [1]. Although *S. aureus* is naturally sensitive to almost all antibiotics, it achieves resistance to antibiotics often due to horizontal gene transfer from external sources. However, chromosomal mutations and types of antibiotics are also involved in this process [2]. Methicillin-resistant *S. aureus* MRSA (Methicillin-Resistant *S. aureus*: MRSA) is considered a severe threat for nosocomial infections and develops problems in remedying bacterial infections. MRSA strains were reported in European hospitals only one year after introducing methicillin in 1961 [3, 4]. Now, these strains have spread all over the world. The prevalence of MRSA strains in Asian countries such as China, Korea, and Taiwan is more than 70%; in North America, it is more than 50%; in Europe is 20%, and it is about 50% in Iran [5, 6]. According to reports, patients with MRSA infection are hospitalized longer than those infected with (Methicillin MSSA Sensitive *S. aureus*); therefore, in addition to the higher cost of treatment, the development of bacteremia or endocarditis happens more often [7]. Infection complications, such as renal failure among patients infected with MRSA, are more than among patients infected with MSSA [8].

Furthermore, even mortality levels among MRSA patients are significantly more than patients with MSSA [9]. Methicillin resistance in this bacterium and its association with beta-lactam antibiotics is developed through a chromosomal sequence named Staphylococcal Cassette Chromosome mec (SCC*mec*), which carries the gene encoding penicillin-alternative protein, which binds to PBP2a with lower affinity. PBPs are highly regulated beta-lactam resistance genes, and the *mec*A gene expression is controlled by the induction system-inhibitor *mec*R1-*mec*I [10]. Exposure to

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oxacillin leads to gene expression (AGR) suppression through pleiotropic effects due to the activation of PBP2a and *mec*A gene expression [11, 12]. Currently, there are four popular methods for typing MRSA strains based on the characteristics of SCC*mec*. Oliveira and De Lencastre's methods using a multiplex method have identified SCC*mec* type I-IV types [13]. In this method, *the mec*A gene and six different loci on the SCC*mec* gene are detected, and *ccr* genes have been developed based on amplification by PCR [14]. However, using different methods may result in different results for MRSA strains with a specific SCC*mec* type. Using the method of Zhang and colleagues, the types of SCCmec type I-V are identified through the multiplex method. This method identifies mecA and a locus on SCC-mec [15]. In the Chongtrakoo *et al*. method, a new classification for naming SCC*mec* has been presented based on the characteristics of the *ccr* genes (which are shown as a number) and *mec*A complex (that is shown as a word) [16]. Considering the high potential of MRSA strains for acquiring antibiotic resistance, as well as spreading and dispersing them in different parts of the hospital, and to improve our knowledge about the control of the source of *S. aureus's* nosocomial infections, this study, the rates of dispersion and reversal of *S. aureus* strains were studied in different parts of Mostafa Khomeini Hospital in Ilam.

2. MATERIALS AND METHODS

2.1. Sampling

The study used descriptive cross-sectional sampling from the air, surfaces, personnel, and patients in seven steps fortnightly in the morning of the working shift from 10 different units of Mostafa Khomeini Hospital. Samples were withdrawn from surfaces, units, and the anterior nasal area of personnel by sterile swab, impregnated with the BHI media. All staff sampling stages were performed with informed consent and under the supervision of the Ilam University of Medical Sciences ethics committee. Air sampling was conducted using an Anderson pump and was done from surfaces at peak hours (9 am to 11 am), and samples of patients were received from the laboratory of the hospice but were again examined in the microbiology laboratory at the Medical Sciences University, Ilam. Here, samples of *S. aureus* were confirmed for phenotype and genotype and then sequenced using *spa* typing and MLST typing methods.

2.2. Sampling of Personnel

Simultaneous samples from different hospital units and samples from personnel's noses were taken (Sampling from the nose including nasal swab 2-3 cm dipping and turning it) and hands (among the fingers and under nails) using sterile swabs dipped in broth BHI media. The staff was volunteers, and the infection control authority completed a questionnaire for all participants that included age, gender, job category, and work experience before sampling. Swabs were placed in 5ml BHI media for growth and, after 48 hours of incubation, were streaked on nutrient agar media (four areas) and were incubated for 18- 24 hours. Gram staining, catalase, and coagulase were used to identify *S. aureus* [17]. Samples were cultured in Mannitol salt agar, and sensitivity to novobiocin disc and tube coagulase DNase test was also performed for final confirmation. The forward PCR primers of TTCAAAAAGGGGACGAATCA and reverse of ACCGTTTCTGGCGTATCAAC for *nuc*A genes designed in GenScript site *spa* (www.spaserver.redom.de) as well as for final approval of encoded *fem*A and *mec*A genes by the strains of *S. aureus* and MRSA was performed [14].

2.3. Sampling of Air

Equipment used in this phase includes an Anderson portable sampling pump, a model book made in Germany, and a Teflon filter Helder with a diameter of 47 mm. The culture media used included Tryptic Soy agar and mannitol salt agar medium. To prevent the growth of fungi on culture media (100 ml) of antibiotic cycloheximide of concentration (5 mg) was also added. Before sampling, all equipment was washed in disinfectant solution (70% alcohol) and then rinsed for 20 minutes in the autoclave at the standard temperature and pressure [18]. Then all equipment was transported to the hospital within sterile packages. In each hospital unit, a series of samples were prepared, and samples were taken. An inflow rate of 28.3 litres per minute was used to determine airflow passes through the filter and sampling time after pre-trial and considering bioaerosols' recommendations of the American Conference of Governmental Industrial Hygienists (ACGIH) [19]. A sampling time of 15 minutes was used for all units, except ICU, CCU, NICU, and operating room units, for which 20 minutes was used. A sampling Pump was placed in the various units or near the patient's bed at the height of one meter from the ground for sampling. During the sampling, each plate was labelled (Code for sample, hospital name, the name of the unit, the sampling unit). After the sampling, the media were transferred to the medical school and placed at a temperature of 37°C. After 48 hours, the total number of bacteria was counted in the Tryptic soy agar and mannitol salt agar plates. The number of colonies grown on media was reported in the number of colonies counted per cubic meter of air to evaluate the total number of bacteria. After determining the number of bacterial colonies, the density was reported as CFU / m^3. The volume of air sampled was obtained using the following equation. $Q = V/T$ [20].

In this regard, the Q index represents the entry flow rate in litres per minute, V shows the obtained air volume in litres, and T represents the time required for sampling. After calculating the standard sampling volume and the number of colonies, the concentration of bioaerosols in $1m^3$ of air was calculated through a simple proportion and reported in CFU/m^3. For a bacteriological survey, colonies grown on mannitol salt agar were examined in terms of the shape and colour of the colony. In order to evaluate the microscopic shape of bacteria, a smear was taken from each sample to provide warm slides and finally were used in the study after confirming the phenotypic and genotypic testing.

2.4. Sampling from the Surfaces

Surfaces were obtained using a sterile swab dipped in BHI broth medium and sterile swabs soaked in saline. The swab dipped in BHI medium broth was wiped over an area of 5×5 cm^2 and then placed into 5ml BHI medium. After 48 hours of incubation for growth, it was incubated for 18- 24 hours on

nutrient agar medium and mannitol salt agar as streaking (4 areas) and then incubated for 48-24 hours. After the growth of bacteria on nutrient media and mannitol salt agar plates, the number of colonies in both plates was counted, and the number of colonies per square meter (CFU / cm^2) was reported. Then *S. aureus* colonies were isolated after the phenotypic and genotypic confirmation was used. The surfaces for sampling included Ventilator, Bed, PlayStation, phone, keyboard, refrigerator, monitoring, floor, trolley, covers cases, Incubators, electric shock instrument, infusions, sinks, medicine cabinet, and suction device.

2.5. Sampling from the Patients

Samples were initially analyzed at the hospital laboratory before being studied for *S. aureus* in the University of Medical Sciences microbiology laboratory. Finally, the samples were used after confirming the *S. aureus* strain and the presence of *spa*, *nuc*A, and *fem*A genes and strains of MRSA with *the mec*A gene.

2.6. Statistical Investigation

Statistical analyzes were carried out using the software spss20. The Chi-square test and the correlations of the results were calculated.

2.7. Antibiotic Vulnerability Testing

The disk diffusion method was performed according to CLSI guidelines. Antibiotics such as gentamicin (GM), linezolid (LZD), doxycycline (DOX), minocycline (MN), ciprofloxacin (CIP), rifampin (RIF), Synercid (SYN), sulfamethoxazole-trimethoprim (SXT) and cefoxitin (FOX, CX). Inducible clindamycin resistance was evaluated by placing clindamycin and erythromycin at a distance of mm 20 -12 from each other. The agar dilution technique defined vancomycin antibiotics' minimum inhibitory concentration (MIC) and oxacillin [21].

2.8. Molecular Typing

*2.8.1. SCC*mec *Typing*

Methicillin resistance is caused by the expression of the *mec*A gene, present in the staphylococcal cassette chromosome (SCC*mec*). For this purpose, the SCC*mec* method was used to identify methicillin-resistant strains. Briefly, a volume of 25 microliters containing 12.5 microliters of master mix (Amplicon, Denmark), 20 µl of each primer and five microliters of template DNA were used. Process in a thermal cycler (BioRad, T100, USA) using conditions: 1 cycle of 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 60°C (SCC*mec* types I, II, III and V) for 45 s, extension at 72 °C for 1 min with a final extension at 72 °C for 10 min. PCR products were run on a 2% agarose gel, stained with SYBR DNA safe dye, and then visualized using the gel under UV light Documentation System (Basic Sciences, Taiwan).

2.8.2. Spa Typing

Protein A is a 49 kDa surface protein originally found in the cell wall of the bacteria *S. aureus*. The spa gene encodes it, and its regulation is controlled by DNA topology. For this purpose, PCR was conducted for the x polymorphic region of A methicillin-resistant protein of *S. aureus* using forward and reverse primers, which were described previously (www.spaserver.redom.de) to *spa* Typing. In addition, the double-stranded gene sequenced PCR products of the spa gene. Finally, the type and sequence of repetitive sequences as a result of the comparison of available sequences with *spa* server repeats (www.spaserver.redom.de) and *spa* typing of each isolate were determined.

2.8.3. MLST Typing

Multilocus sequence typing (MLST) is used to sequence DNA fragments and characterize bacterial species isolates (typically about 500 bp) from seven housekeeping genes. The sequences of these genes are compared with the known alleles at each location through the MLST website (http://www.mlst.net), the results obtained from each isolate are checked with a seven-allele profile, and finally, a type of sequence (ST) defines. For this purpose, 36 MRSA isolates were selected for MLST typing. To study the genetic variations in the alleles of seven housekeeping genes of MRSA strains by MLST, Enright, and colleagues in http://saureus.mlst.net site was used [22].

3. RESULTS

In total, 137 strains of *S. aureus* were isolated from various units of the hospital, including 76 (55.48%) from surfaces (65 isolates by BHI and 11 strains of normal saline), 32 (36.23%) from staff, 19 (86.13%) from the air, and 10 (30.7%) from patients.

3.1. The Results of the Evaluation of Hospital Staff in Terms of the *S. aureus* Carriage

Our results showed that 32 subjects (23.36%) were carriers of *S. aureus*. Among these, 24 cases (75%) were carriers of *S. aureus* in the nose 8 (25%) of subjects carried *S. aureus* on their hands.

Based on the occupational group, subjects included 23 nurses (71.9%), five health workers (15.6%), and four service staff (12.5%) ($P<0.34$). Personnel with work experience of more than ten years had the highest carrier rates ($P<0.56$). The most significant carriers were related to the CCU (7 persons, 12.9%), where emergency and operating room staff with the high similarity (15.6%) had the most significant number.

3.2. Screening the Staff's Transporters of *S. aureus* in the Nose

Twenty-one staff transporters of *S. aureus* in the nose were introduced to infectious units for antibiotic treatment. Nine cases were treated using mupirocin ointment, and after this time, they were again sampled using the swab from the nose of staff, from which *S. aureus* was not isolated on re-examination.

3.3. The Results of the *S. aureus* Strains were Isolated from Clinical Specimens of Patients

Ten strains of *S. aureus* were obtained from clinical instances of patients admitted to the hospital at the time of

sampling. The prevalence of *S. aureus* in clinical samples of blood (60%), urine (20%), ulcers (10%), and chips (10%) were reported.

3.4. Results of the Relative Abundance of Colony Count of Bacteria Isolated from Surfaces

76.91 CFU / cm^2 of bacteria from various hospital surfaces were isolated, from which 11 strains of *S. aureus* were used. The highest bacteria counts were isolated from a wheelchair patient (13.6CFU / cm^2) and the floor (13/22 CFU / cm^2).

3.5. Results of Studying the *S. aureus* Strains Isolated from the Surfaces

A total of 330 samples were isolated from surfaces using a swab dipped in BHI medium, from which 65 strains of *S. aureus* were obtained. The highest *S. aureus* count was obtained from the surfaces of CCU (16.92) and the women's health unit (13.84%), and the lowest count was obtained from the post-CCU unit for men and women.

3.6. Results of Counting the Total Average of Colony Counting and Bacteria in Air Samples

From 8 sampling stages from the air, 1572 colonies were isolated. Nineteen colonies were confirmed to contain *S. aureus*. The total average of bacteria counts in different units showed that the women's health unit (119.6 CFU/m^3) had the highest, and ICU had the lowest (28.9 CFU/m^2). November, the highest bacteria counts were also achieved (33.82CFU/m^2). The average specific enumeration of *S. aureus* based on CFU/m3 showed that the women's health unit (21.05%) and emergency unit (15.78%) had the highest number of *S. aureus*.

3.7. Results of *S. aureus* Antimicrobial Susceptibility Using Disk Diffusion Method

Unfortunately, for unpredictable reasons, from 137 isolated strains of *S. aureus*, only 88 remained for future studies, and the rest were destroyed. Meanwhile, 36 (40.9%) strains were resistant to cefoxitin, and 52 (59.1%) isolates were susceptible to cefoxitin. The lowest antibiotic resistance is also related to gynaecological surgery and post CCU in men. Table **1** shows the results of antibiotic resistance in isolates of methicillin-resistant *S. aureus* and cefoxitin disk, the strain isolated from the CCU and NICU.

In total, the highest number of MSSAs isolated were related to the sample from female staff, surface samples (bed), and air samples 10 (11.4%), 8 (9.1%), and 7 (8%), respectively. The highest number of MRSA isolates was related to the female staff, which were isolated from air and surface samples with the numbers of 10(11.4%), 8(9.1%), and 7(8%), respectively. The results of the sensitivity of the isolated strains of *S. aureus* are shown separately in Table **2**. Among the selected isolates of *S. aureus*, 39 strains (44.32%) were sensitive to clindamycin, and 27 were erythromycin-resistant. Nine of the 22 strains susceptible to clindamycin and erythromycin resistance had a D-induced phenotype. According to Table **3**. The highest number of strains susceptible to clindamycin was MSSA (51.92%, 27.52). In contrast, the highest number of strains resistant to erythromycin was related to the MRSA strains, with a frequency of (83.33%, 30.36).

Based on the minimum inhibitory concentration (MIC) results, none of the isolated Staphylococcus aureus strains was resistant to vancomycin. 27.3% of S. aureus strain had a MIC of 16-8 µg, 11.4% had a MIC of 64-32 µg and 5.7% and MIC of > 64µg to oxacillin.

Table 1. Relative frequency of antibiotic resistance in methicillin sensitive and resistant strains isolated 88 Mostafa Khomeini hospital.

Antibiotics	Resistance	Moderate	Sensitive	Total
				n =88
Cefoxitin	36 (%40.9)	0(%0)	52(%59.1)	88 (%100)
Doxycycline	16(%18.2)	13(%14.8)	59(%67)	
Clindamycin	25(%28.4)	6(%6.8)	57(%64.8)	
Erythromycin	47(%53.4)	2(%2.3)	39(%44.3)	
Gentamicin	40(%45.50)	6(%6.8)	42(%47.7)	
Synercid	1(%1.1)	1(%1.1)	86(%97.8)	
Linezolid	4(%4.5)	0(%0)	84(%95.5)	
Minocycline	6(%6.8)	8(%9.1)	74(%84.1)	
Ciprofloxacin	12(%13.6)	8(%9.1)	68(%77.3)	
Trimethoprim-sulfamethoxazole	17(%19.3)	0(%0)	71(%80.7)	
Rifampin	13(%14.8)	0(%0)	75(%85.2)	

Table 2. Results of frequency of sensitivity to methicillin in isolated strains of *S. aureus.*

Sample Type		MRSA	MSSA	Total
Female Personnel	Personnel	0(0%)	10(11.4%)	10(11.4%)
Male personnel		4(4.5%)	6(6.8%)	10(11.4%)
Case cover	Surfaces	1(1.1%)	3(3.4%)	4(4.5%)
Computer		1(1.1%)	1(1.1%)	2(2.3%)
Icebox		3(3.4%)	2(2.3%)	5(5.7%)
Trolley		2(2.3%)	0(0.0%)	2(2.3%)
Electroshock		0(0.0%)	1(1.1%)	1(1.1%)
Bed		3(3.4%)	8(9.1%)	11(12.5%)
Floor		1(1.1%)	1(1.1%)	2(2.3%)
Station		5(5.7%)	4(4.5%)	9(10.2%)
Sink		1(1.1%)	0(0.0%)	1(1.1%)
Ventilator		1(1.1%)	0(0.0%)	1(1.1%)
Monitoring		1(1.1%)	0(0.0%)	1(1.1%)
Phone		1(1.1%)	2(2.3%)	3(3.4%)
Medicine cabinet		2(2.3%)	1(1.1%)	3(3.4%)
Incubator		1(1.1%)	0(0.0%)	1(1.1%)
Personnel mobile		1(1.1%)	0(0.0%)	1(1.1%)
Aspirator		2(2.3%)	0(0.0%)	2(2.3%)
Urine sample	Patient	0(0.0%)	1(1.1%)	1(1.1%)
Wound culture sample		0(0.0%)	1(1.1%)	1(1.1%)
Chip sample		0(0.0%)	1(1.1%)	1(1.1%)
Blood sample		1(1.1%)	3(3.4%)	4(4.5%)
Air	Air	5(5.7%)	7(8%)	12(13.6%)
Total		36(40.9%)	52(59.1%)	88(100%)

Table 3. Induced resistance phenotype in susceptible and methicillin-resistant strains isolated.

Strain Phenotype	MRSA n=36	MSSA n=52	Total
Clindamycin (Sensitive), Erythromycin (sensitive)	12 (%33.33)	27 (51.92)	39 (%44.32)
Clindamycin (Resistant), Erythromycin (Resistant)	18 (%50)	9 (17.30)	27 (%30.68)
Clindamycin (Sensitive), Erythromycin (Resistant)	4 (%11.11)	9 (17.30)	13 (%14.77)
Clindamycin (Sensitive), Erythromycin (Resistant)	8 (%22.22)	1 (1.2)	9 (%10.23)

Table 4. The results of molecular typing (SCC*mec* Typing).

Ward	SCC*mec*					Total
	I	II	III	IV	V	
Post CCU Men	0	0	0	3	0	3
Men	0	0	1	2	0	3
Women	0	0	2	0	1	3
ICU	0	1	2	1	0	4
CCU	2	2	2	5	0	11
NICU	1	1	0	3	0	5
OR	0	2	0	0	0	2
ER	0	0	1	0	0	1
Women Surgery	0	0	2	2	0	4
Total	3	6	10	16	1	36

3.7.1. PCR Results

In the PCR test on 36 strains resistant to hard Cefotaxime, *the mec*A gene was identified in all isolates. The strains isolated from the surfaces had this gene's highest (72.2%) frequency. In contrast, strains isolated from the patients (2.78%) had the lowest frequency of the *mec*A gene. Among the studied units, CCU and NICU, with frequencies of 31% and 14%, had the highest frequency of the *mec*A gene, respectively. The post CCU of women did not have this gene ($P > 0.673$).

3.8. The Results of Molecular Typing (SCC*mec* Typing)

From 36 strains of MRSA, 16 cases (44.4%) had IV SCC*mec* type strain, ten strains (27.8%) had SCC*mec* type III, six strains (16.67%) were II SCC*mec* type, three strains (8.33%) were SCC*mec* type I, and one race (2.78%) was V SCC*mec* type, among which typing of SCC*mec* type IV (44%) was more common (Table **4** shows the relative frequency of SCC*mec* type frequency among the different samples). 36.11 % of MRSA belonging to SCCmec type IV were resistant to erythromycin and gentamycin. Also, 22.22% of MRSA belonging to SCCmec type III were resistant to clindamycin and trimethoprim-sulfamethoxazole.

In contrast, the other type was sensitive to all antibiotics.

3.9. The Results of Molecular Typing (*spa* Typing) of *S. aureus* Strains

Investigation of the variable section of gene encoding protein A, 14 *spa* typing of 36 separates of *S. aureus* resistant to methicillin (MRSA) showed that 72.2% of samples were isolated from the surfaces and 13.9% of samples were isolated from air, and 11.2% were isolated from staff. 037 t 19.4% was the most prevalent, followed by 030t (16.7%), and 701t (11.1%) were the most frequent types. Additionally, three new types, 15471t, 15474t, and 17470t, were also identified among the strains recorded and named Iran in the http://*spa*server2.ridom.de server. 30.6% of the identified types were related to the CCU, 50% of types were associated with 030t, and most isolated types were obtained from the station surface (13.9%) and the samples of males (11.1%) (Table **5**). t030, most isolated from the hand and nose of the staff, was identified among multi-drug resistance MRSA. t701 was isolated from the surfaces and air, but the isolates typed t037 were found only on the surface samples. Meanwhile, the *spa* type t030 and t701 had the highest IV SCC*mec* type, but *spa* type t037, related to SCCmec type III, was the most frequent isolated type. New *spa* types of t15471 and t15474 were related to IV SCC*mec* type, and the new *spa* type of t17470 was associated with SCC*mec* type II, while the only train with V SCC*mec* type belonged to t078 *spa* type.

Among the strains of MRSA, 11 different sequence types were found. ST type 239 (10.36, 27.8%) and ST22 (19.44%) and ST6 and ST30, each with 11.11%, were the most detected type sequences. *Spa* type 037 with the ST239 and t030 with sequence types 239, 291, 246, 701t with ST6 and three new types 15471, 15474, and 17470 were associated with the ST22. Among the 36 strains of MRSA, 27 isolates (75%) belong to clonal complex 329, and 9 strains (25%) were related to clonal complex 22, respectively. Types t037, t030, and t701 were related to complex clonal 329CC, and three of the new types of t15471, t15474 and t17470 were related to complex clonal CC22, respectively that 12 strains of clonal complex 329 were related to IV SCC*mec* type (12.72, 44/44%) (Table **5**).

4. DISCUSSION

Methicillin-resistant *S. aureus* has been introduced as a primary hospice pathogen and is expanding in various human societies. However, it is not clear whether the strains of a unit of the hospital are related to other units and whether the subspecies having these genes share these resistance genes with other species or not. Most strains of methicillin-resistant *S. aureus* have one of three resistance cassettes (SCC*mec*) in their chromosomes [23]. In this study, 32 patients (23.36%) of the total staff (120) carried *S. aureus* in the nose (n = 24, 75%) and hands (n = 8, 25%), which compared to a similar survey in Iran in 2011 with 19.2% and 15.9% carrying *S. aureus* in nose and hands [24]. Although nurses (71.9%) and service staff (12.5%) had the highest and the lowest rate of *S. aureus* in their nose, similar to previous studies, no relationship was found between the job and the amount of *S. aureus*. The personnel in the CCU had the highest frequency of *S. aureus* on their hands (9.4%) and in the nose (21.9%).

Table 5. Clinical and molecular analysis of MRSA isolated from different origins and ward of the hospital.

No	Sample	Ward	SCC*mec*	*spa*	ST	CC
1	Refrigerator	Men	III	t037	239	239
2	Staff=Men	CCU	IV	t030	291	239
3	Ventilator	CCU	IV	t030	291	239
4	Staff=Men	Post CCU Men	IV	t15474	22	22
5	Staff=Men	CCU	II	t1414	30	239
6	Staff=Men	CCU	IV	t030	239	239
7	Monitor	CCU	I	t325	859	239
8	Medicine cabinet	NICU	IV	t030	239	239
9	Bed	Women Surgery	IV	t15471	22	22
10	Station	NICU	I	t275	30	239
11	Station	CCU	IV	t15471	22	22
12	Mobile staff	Women Surgery	IV	t790	22	22
13	Air	CCU	I	t275	30	239
14	Air	CCU	II	t325	867	22
15	Patient=BC	ICU	II	t325	867	22
16	Aspirators	CCU	IV	t701	6	239
17	Station	OR	II	t1358	889	239
18	IPhone	ICU	III	t037	239	239
19	Bed	Women	III	t037	239	239
20	Refrigerator	Women	III	t037	239	239
21	Computer	Women Surgery	III	t037	239	239
22	Refrigerator	Men	IV	t12498	1465	239
23	Station	NICU	IV	t12498	1465	239
24	Medicine cabinet	OR	II	t1358	859	239
25	Air	NICU	II	t15470	22	22
26	Air	Post CCU Men	IV	t701	6	239
27	Cover records	ICU	IV	t030	246	239
28	Floor	Women Surgery	III	t037	239	239
29	Trolley	Men	IV	t701	6	239
30	Trolley	CCU	III	t 275	30	239
31	Incubator	NICU	IV	t030	239	239
32	Sink	ICU	III	t037	239	239
33	Aspirators	ER	III	t 14870	22	22
34	Air	Post CCU Men	IV	t701	6	239
35	Station	Women	V	t 078	26	239
36	Bed	CCU	III	t 14870	22	22

In contrast, bacteria were not detected on the hands of ICU and NICU staff and in the nose of the post CCU unit of Women. The differences in the frequency of the carriers of *S. aureus* in our study compared to other studies could be due to the number of samples, place of sampling, and the health control personnel in various studies [25]. Due to the type of patients admitted to the CCU and the presence of carriers as a potential reservoir of infection, studying the prevalence of carriers and using straightforward methods, such as frequent washing of hands with soap or antiseptic ointment mupirocin nasal 2% or antibiotic treatment to eradicate the carriers can help for eradication [26]. This study treated staff infected in different hospital units with mupirocin ointment. After re-sampling from the nose, no *S. aureus* was isolated, which can prove this. A comparison of our results with a survey conducted in 2012 in Iran was close, showing that (91.1%) of *S. aureus* was sensitive to linezolid and Synercid. However, in the same study between 2008 and 2012 in other hospitals in

Iran, linezolid susceptibility percent was calculated as (100%), which was higher than our results [27, 28].

Disc diffusion usually shows false-negative results in tests in different antibiotic resistance methods and mainly reports its sensitivity more than the heterogeneous low-resistance strains [29, 30]. The results indicate that the widely performed disk diffusion test or antibiogram in diagnostic laboratories can also cause false-positive results. The amount of inoculum, medium diameter, and used discs can all affect the results. Agar disk diffusion screening test and cefoxitin and Moxalactam discs are alternative methods reported in the literature and used in this study [29, 31]. In this study, among the 36 strains of MRSA, five different types of SCC*mec* type were detected; among them, SCC*mec* type IV (44%) was more common, and the type of SCC*mec* type III, II, I, and V had the frequencies of (27.8%) (16.67%) (8.33%) and (2.78%). The *spa* type t030 with a frequency of 6 strains had the highest type IV SCC*mec,* but seven strains with *spa* type t037 associated with SCC*mec* type III were the most frequent isolated types. In addition, new *Spa* types of t15471 and t15474 were related to the IV SCC*mec* type, and new *spa* type of t17470 was associated with SCC*mec* type II, and V SCC*mec* type strains belonged to the t078 *spa* type. In similar studies in Iran, ST239 with 82% and China with 97% as ST type were the most common that were associated with the SCC*mec* type III and in our study found with 19.44% (7.36) in type III was found that the most common ST and SCC*mec* type of hospital was reported [32, 33]. One study showed 90% of HA-MRSA in Asia with 239 matched ST [34]. Data shows that in Asian countries, a possible spread of ST30 is expected between different countries [35]. The study also proved that these clones (Table **5**). However, as expected, ST8 (USA300) is the most common CA-MRSA clone colon or ST80 clone in the United States of America and Europe; as expected, so far not been seen in any Asian country nor found in our study. Although a small number of infections with ST8 CA-MRSA have recently been reported in Japan [36], South Korea [37] or ST80 infection with CA-MRSA has been reported in Singapore [38] and Malaysia [39], indeed, the clone ST8 and ST80 MRSA in Asian countries are rare [35]. Similar to our study, Kinnevey *et al*. report resistant to methicillin between 2000 and 2012 from patients in different hospitals. They showed that t190 had a frequency of (17.4%) t878 with (7.6%), and t032, which had a frequency of (5.4%). Genetic exchange between different strains of MRSA may lead to the emergence of resistant strains with new features that, according to the results obtained, more control over clones resistant to MRSA strains and existing reservoirs should be considered [40]. In a similar study, *Spa* types t030, and t037, with 22.2% and 33.3%, respectively, show higher frequencies than our results [41]. Similar to type t030 in our study was reported in other studies in China [42] and Iran in 2014. A similar study in 2014 showed that type 701t was isolated from Western country samples that probably represent the frequency of this type in the West [28, 43]. Www.*spa*server.redom.de, types including t003, t032, t002, t008, and t011 were introduced as five *spa* types among almost 10000 types, while these *spa* types were not observed in the results obtained in the present study. In our study, the most typed isolated samples were ST239-*spa* t037- SCC*mec* III ST239-*spa* t037-*SCCmec* III. For the first time in 2009 was reported by 83.3% of Malaysia [44]. In a study on a sample in 2005-2010 in China, ST239-*spa* t037-*SCCmec* III MRSA was the most identified clone [45, 46]. According to these results, this could be a typical clone in Asian hospitals. A similar study in Denmark indicates that 84% of the strains were isolated to the colon ST80-IV. An increase in MRSA clones in such communities as Denmark needed more control, especially in conjunction with hospital staff at risk of infection, because if they are not controlled, it can threaten the general population's health [47]. In another study, Hoon Sung *et al*. studied the prevalence of *S. aureus* resistance to methicillin in communities and hospitals in eight Asian countries. The results showed that 4117 samples were isolated from patients, of which 1463 were related to infections. The number of strains isolated from people in 2162 (52.5%) was included. Of 25.5% of methicillin-resistant strains isolated from society, 67.4% were distributed among isolates from hospitals.

The most common clones isolated in communities and hospitals included ST59, ST30, and ST72. The findings show the different clones of MRSA between communities and hospitals35. Based on the MLST results obtained in the study, among the MRSA strains, 11 different typing sequences were found ST239 (27.8%) and ST22 (19.44%) and ST6 and ST30 each were identified with (11.11%) the highest type sequence. T037 was associated with ST239 and t030 with the ST239, ST291, and ST246 types, t701 with ST6, and three new types of t 15471, t 15474, and t 17470 with ST22. In similar studies in Iran and China, ST239 was 82% and 97%, respectively, as the most common ST associated with SCC *mec* type III. In the present study, ST239 with 19.44% (36.6) had the highest association with SCC *mec* type III, which was reported as the most commonly reported hospital-type sequence [32, 33]. In a study, 90% of hospital-acquired infections in Asia were consistent with ST 239 [34]. Sequences Type 22 were isolated from staff and surfaces, with the 22 and 859 sequence sequences reported in the same study in Iran in 2013. Like Kinnevey PM, ST22 is endemic in the Irish region[48, 49]. In a study, 90% of hospital-related infections in Asia were consistent with ST 239 [34]. Type 22 sequences were distinguished from staff strain and levels, with the 22 and 859 sequences reported in the same study in Iran in 2013. In the Irish region, ST22 is endemic, and in our study, consistent with Kinnevey PM, this type sequence was also isolated [48, 49].

CONCLUSION

According to the results obtained in this study, resistant clones in hospital units could penetrate other sectors and their people. According to the results obtained in this study, resistant clones in hospital units could penetrate other sectors and their people. Staff in different units can transfer the clones into the other units and individuals and underlie the frequency of nosocomial infections and more severe infection epidemics in society. Therefore, some measures must be adopted to control the movement of personnel to different units in command to prevent the spread of resistant clones to the other units and individuals in the community.

LIST OF ABBREVIATIONS

MIC	=	Minimum Inhibitory Concentration
MLST	=	Multilocus Sequence Typing
MRSA	=	Methicillin-Resistant *S. aureus*
PCR	=	Polymerase Chain Reaction

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The current research project with code; 931022/47 has been approved by Ilam University of Medical Sciences as a student dissertation and this university has provided research support.

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or research committee and with the 1975 Declaration of Helsinki, as revised in 2013.

CONSENT FOR PUBLICATION

Informed consent was obtained from all participants. A sampling of hospital staff was done with informed consent and under the supervision of Ilam University of Medical Sciences (ethics committee code 931022/47).

AVAILABILITY OF DATA AND MATERIALS

The data and supportive information are available within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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